ARTICLE

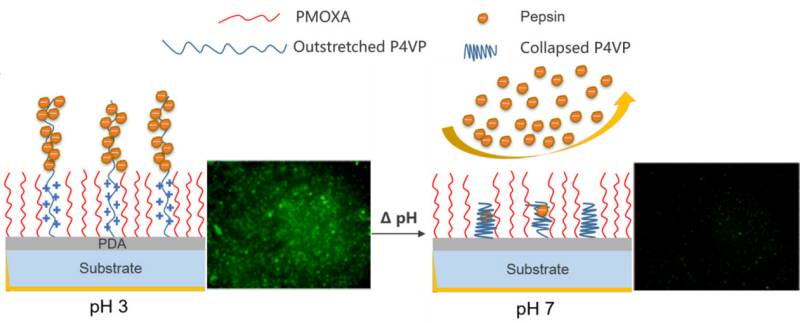
Polydopamine Anchored Poly(2-methyl-2-oxazoline)/Poly(4-vinyl pyridine) Mixed Brushes with Switchable Properties for Pepsin Adsorption

Kang He, Fei Hu, Chaoshi Chen, Muhammad Atif, Yanmei Wang*

CAS Key Laboratory of Soft Matter Chemistry, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei 230026, China.

(Dated: Received on June 21, 2021; Accepted on September 27, 2021)

Mixed polymer brushes coating based on poly (2-methyl-2-oxazoline)/ poly(4-vinyl pyridine) (PMOXA/P4VP) was prepared by simultaneously grafting amine-terminated PMOXA and thiol-terminated P4VP onto poly(dopamine) (PDA)-modified substrates in this work. The coatings were characterized by X-ray photoelectron spectroscopy, ellipsometry, zeta potential measurements, and the static water contact angle tests. The results indicated that it is feasible to control the components of the coating by adjusting the feed ratio of PMOXA to P4VP. Moreover, the zeta potential and the water contact angle of mixed brushes modified surfaces could be tuned by changing the environmental pH value and surface compositions. Finally, fluorescein isothiocyanate-labelled pepsin assay and surface plasmon resonance were performed to investigate the responsive adsorption/desorption of pepsin by PMOXA/P4VP mixed brushes. The results showed that by adjusting the fraction of PMOXA or P4VP, the PMOXA/P4VP mixed brushes coated surfaces could adsorb a high amount of pepsin at pH=3, and achieve a desorption efficiency of over 92% at pH=7.



Key words: Binary mixed brush, Zeta potential, Controlling protein adsorption, Pepsin, Surface plasmon resonance

I. INTRODUCTION

Controlling protein adsorption/desorption is a pressing issue in many biotechnology fields, such as biosensors, medical device coatings, and drug delivery, *etc.* [1−5]. Biomaterial surfaces modified with stimuli-responsive polymer brushes that show the ability to control protein adsorption and desorption are thus highly desired [6, 7]. However, the protein-interface interactions are quite complex and diverse, involving van der Waals forces, electrostatic interactions, hydrophobic interactions, and conformational entropy [8−12]. Therefore, it is difficult for surfaces modified with stimuli-responsive polymer brushes alone to realize the controlled or switchable protein adsorption [13−16].

In order to realize the controlled protein adsorption, mixed polymer brushes composed of polymer with anti-fouling property and stimulus-response polyelectrolyte have been proposed. Bratek-Skicki has fabricated the mixed poly(ethylene oxide) (PEO) and poly(2-(dimethylamine)ethyl methacrylate) (PDMAEMA) brushes [17], in which PEO plays an anti-fouling part, the PDMAEMA as the stimulus-response polyelectrolyte could be responsive to pH and ionic strength

*Author to whom correspondence should be addressed. E-mail: wangyanm@ustc.edu.cn

change. It was demonstrated that human serum albumin (HSA) and human fibrinogen adsorbed on mixed PEO/PDMAEMA brushes could be completely desorbed when changing pH and ionic strength, while on PDMAEMA homobrushes, only 61.0% of adsorbed HSA and 23.0% of adsorbed human fibrinogen could be released. Dupont-Gillain *et al.* have investigated the adsorption/desorption capability of HSA on mixed brushes of PEO/poly(acrylic acid) (PAA) induced by pH and ionic strength [18]. It is observed that the PEO/PAA mixed brushes are able to achieve a desorption percentage of 86% after protein adsorption, displaying a great improvement of desorption percentage compared with PAA homobrushes (desorption percentage is only 30%). It can be concluded that the introduction of protein repellent PEO could effectively compensate for the imperfect protein desorption efficiency of the environment-responsive polymer.

However, oxidative degradation and enzymatic cleavage of PEO could result in the decrement of stability for PEO coating after its long-term use [19, 20]. Recently, poly(2-methyl-2-oxazoline) (PMOXA) has drawn more attention for its similar protein resistant ability to PEO, while it might be less prone to degradation than PEO [21−25]. Pan *et al.* have prepared PMOXA/PAA mixed brushes through polydopamine (PDA) anchor for bovine serum albumin (BSA) and lysozyme adsorption/desorption [26, 27]. It is demonstrated that the desorption percentage of protein could be improved highly from 50.5% for PAA homobrushes alone to 87.0% for PMOXA/PAA mixed brushes.

Besides PAA, poly(4-vinyl pyridine) (P4VP), a kind of weakly basic polyelectrolyte, has been extensively investigated for its pH-dependent protonation of its pyridine ring along the chain and the consequent pH-dependent swelling-deswelling behavior [28−30]. When pH value of environment is lower than the isoelectric point of P4VP (pI≈4.8) [31], the electrostatic repulsion between positively charged repeating pyridine groups produces a certain degree of swelling of the brush; when pH value is higher than pI value of P4VP, polymer chains collapse due to the neutralization of pyridine groups, as well as the hydrophobic interaction between vinyl groups existing in polymer chains [32, 33].

On the basis of the swelling-deswelling behavior of P4VP upon pH change and the stable anti-fouling property of PMOXA, PDA anchored PMOXA/P4VP binary mixed brushes with the property of controlled protein adsorption were designed in this work to investigate the controlled or switchable protein adsorption. Usually, the pepsin with extremely low isoelectric point (pI≈2.2) is a reliable indicator for the diagnosis of laryngopharyngeal reflux disease [34], therefore in this work pepsin was chosen as a model protein to study the interaction between surfaces and proteins. Firstly, PMOXA/P4VP binary mixed brushes were prepared by simultaneously grafting amine-terminated PMOXA (PMOXA-NH_2) and thiol-terminated P4VP (P4VP-SH) with designed chain length to PDA modified silicon/glass/gold substrates. The coating composition, surface hydrophilicity, thickness, and surface charge were measured by X-ray photoelectron spectroscopy (XPS), static water contact angle (WCA), variable angle scanning ellipsometry (VASE), and zeta potential, respectively. Furthermore, the behavior of pepsin adsorption/desorption on the coatings surfaces was evaluated by fluorescence microscopy and surface plasmon resonance (SPR).

II. EXPERIMENTS

A. Materials

2-Methyl-2-oxazoline (MOXA, 99%, Sigma-Aldrich Chemical Company, USA) and 4-vinyl pyridine (4VP, >95%, Tixiai, Shanghai, China) were dried over calcium hydride overnight and distilled under atmospheric and reduced pressure conditions, respectively. Dopamine hydrochloride, potassium phthalimide, methyl trifluoromethanesulfonate (MeOTf, 98%), potassium chloride (KCl, 99.98%), and pepsin (pI≈2.2) from porcine gastric mucosa were purchased from Sigma-Aldrich and used as received. 2,2-Azobisisobutyronitrile (AIBN, Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China) was recrystallized from ethanol. Tris(hydroxyl methyl)-aminomethane (Tris), hydrazine hydrate, ethanolamine, ethanol, hydrochloric acid (HCl), chloroform ($CHCl_3$), acetonitrile, and isopropyl alcohol were obtained from Sinopharm Chemical Reagents (Shanghai, China). ACN and IPA were dried over calcium hydride for 6 h and distilled before use. S-1-dodecyl-S-(α,α-dimethyl-α-acetic acid) trithiocarbonate (DDMAT) and fluorescein isothiocyanate-labeled pepsin (FITC-pepsin) were prepared according to previous literatures [35, 36]. The gold SPR sensors were received by coating glass slides with an adhesion promoting chromium layer (thickness

DOI:10.1063/1674-0068/cjcp2106103

5 nm) and a surface plasmon active gold (Au) layer (45 nm). Silicon(111) wafers with natural oxidized layer were derived from Zhejiang Crystal Photoelectric Technology Company (Taizhou, China). Glass slides were purchased from Shanghai Jinglun Industry Glass Company (Shanghai, China).

B. Synthesis of the polymers

The typical synthesis process of PMOXA and P4VP is shown in FIG. S1 in Supplementary materials (SM). PMOXA was synthesized by using cationic ring-opening polymerization (CROP) of MOXA with MeOTf as an initiator (the molar ratio of monomer to initiator was 25:1), then end-capped with potassium phthalimide [24]. The successful preparation of PMOXA-NH_2 with a degree of polymerization of 28 was confirmed by ^{1}H NMR spectroscopy (FIG. S2 in SM).

P4VP was synthesized by using reversible addition-fragmentation chain transfer (RAFT) polymerization with AIBN as initiator and DDMAT as a chain transfer agent (the molar ratio of monomer to chain transfer agent was 100:1) [37]. The successful preparation of P4VP-SH with a degree of polymerization of 95 was confirmed by ^{1}H NMR spectroscopy (FIG. S3 in SM). The calculated theoretical contour chain lengths of the PMOXA-NH_2 and P4VP-SH were 10.62 nm and 23.90 nm, respectively (Table S1 in SM).

C. Preparation of polymer brushes

In order to remove any organic contamination on raw substrates, silicon/glass wafers were cleaned by sonication in ethanol, acetone and deionized water for 15 min successively, then immersed in freshly prepared piranha solution (7:3, V/V mixture of H_2SO_4 (95%−98%) and H_2O_2 (30%), 50 °C) for 4 h. The gold SPR sensors were cleaned by freshly prepared piranha solution for 1 h at 70 °C. And then, all substrates were rinsed with an excess of deionized water and dried under nitrogen atmosphere.

Polymer brushes were prepared using the grafting-to method in this work. Firstly, the cleaned substrates were immersed in the freshly prepared Tris-HCl solution (pH=8.5) of dopamine hydrochloride (2 mg/mL) with continuous vibration at room temperature, in contact with atmospheric oxygen. Afterwards, a 10 mg/mL solution of polymers containing PMOXA and P4VP was prepared by dissolving both polymers with the solution containing 30% Tris-HCl (pH=8.5) buffer and 70% ethanol. The mass ratios of PMOXA/P4VP used in this work were 100/0 (PMOXA homobrushes), 80/20, 50/50, 20/80, and 0/100 (P4VP homobrushes), respectively. Substrates modified by PDA were soaked in polymer solutions mentioned above at 50 °C for 24 h, then the samples were rinsed extensively with deionized water and dried by a stream of nitrogen. Corresponding prepared surfaces were named as PMOXA, $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, $PMOXA_{20}/P4VP_{80}$, and P4VP, respectively.

D. Characterization

1. Nuclear magnetic resonance (^{1}H NMR)

The ^{1}H NMR measurements of polymers were performed by using an AVANCE 300 spectrometer (Bruker biospin, Switzerland) (300 MHz) at room temperature.

2. XPS

The chemical composition of the polymer modified silicon substrates was investigated by a VG ESCALAB MK II X-ray Photoelectron Spectrometer (XPS, VG Scientific Instruments, East Grinstead, England) with an Al Kα X-ray source (energy of 1486.6 eV) at room temperature. The take-off angles of the photoelectrons were set to 90° and the spot size was kept at 500 μm.

3. VASE

The dry thicknesses of brushes coating on the silicon wafer were measured using a variable angle spectroscopic ellipsometer (M-2000, Woollam Co., Inc., Lincoln, NE) at room temperature. The ellipsometric data were acquired in the spectral range of 370−1000 nm at two different angles of incidence (65° and 75° for silicon wafer). The analysis software, CompleteEASE 4.81, was used to analyze all data. To fit the ellipsometric data, the optical constants (refractive index, extinction coefficient) of Si (n=3.865, k=0.020) and SiO_2 (n=1.465, k=0) were used to determine the SiO_2 layer thickness of the freshly cleaned silicon surfaces. Each polymer layer was represented as a slab of uniform thickness having sharp interfaces and optical properties described by a Cauchy model. The average thickness value was obtained by measuring one sample in at least three different positions, and the data were reported as means±standard deviation (SD).

DOI:10.1063/1674-0068/cjcp2106103

4. Zeta potential

The surface zeta potential of the samples was investigated by SurPASS electrokinetic analyzer (Anton Paar GmbH, Graz, Austria). The pH of the KCl (0.001 mol/L) solution was adjusted from 7 to 3 by the addition of hydrochloric acid (0.05 mol/L). The average surface zeta potential value was recorded by measuring one sample in at least three times, and the data were reported as means±SD.

5. WCA

The surface hydrophilicity of the coating surfaces was measured by Optical Contact Angle & Interface Tension Meter SL200KS (SOLON TECH, Shanghai, China, manufactured under authorized from USA KINO Industry Co. Ltd.). Before measurement, the samples were immersed into aqueous solutions of different pH for 2 h (aqueous solutions with pH=2−9 were prepared by adding 1 mol/L hydrochloric acid or 1 mol/L sodium hydroxide solution into deionized water under the analysis of a pH meter). The average WCA value was obtained by measuring one sample in at least three different positions, and the data were reported as means±SD. Aqueous solutions with pH=2−9 were used in the experiments.

6. Fluorescence microscopy

To evaluate the protein adsorption/desorption property of the coatings, FITC-pepsin was chosen as the model protein. Bare and modified glass wafers were incubated in the FITC-pepsin solution (1.0 mg/mL, hydrochloric acid solution of pH=3) for 3 h in dark, and then the samples were washed with the aqueous solution (pH=3) three times to remove the weakly bound proteins. Subsequently, the samples were immersed in deionized water (pH=7) for another 2 h, then rinsed with the deionized water extensively and dried by a stream of nitrogen. Lastly, the glass wafers were imaged under an optical microscope, Olympus BX81 (Olympus, Japan) equipped with a halogen lamp, filter U-MNG2 (λ_{exit}=470−490 nm, λ_{emit}>510 nm) and camera type DP72. Image J software was used to evaluate the fluorescence intensity from three different positions and the mean values were calculated, with the data expressed as means±SD.

7. SPR

Biacore T200 Instrument (Ge Healthcare Bio-Science AB, Uppsala, Sweden) was used to analyze protein adsorption/desorption quantitatively on the surfaces at room temperature. Firstly, hydrochloric acid solution of pH=3 was flowed through the sensor surface for 600 s to obtain the baseline signal, and then the protein solution (pepsin of 0.1 mg/mL, pH=3) was injected for 900 s for the protein adsorption. After rinsing by pH=3 aqueous solution for 600 s, the deionized water (pH=7) was passed onto the sensor surface for 240 s, and ended with 600 s injection of pH=3 aqueous solution. The flow rate was maintained at 20 μL/min throughout the procedure. The response signal from the SPR measurement was recorded in response units (RU). Generally, 10 response unit equals 1.0 ng/cm^2 of protein adsorbed on the sensor surface.

III. RESULTS AND DISCUSSION

A. Characterization of polymer-modified silicon wafers

In this work, PMOXA/P4VP binary mixed brushes were prepared by "grafting to" method through the Michael addition reaction between the amine end group of PMOXA-NH_2 or thiol end group of P4VP-SH and quinone existing in PDA. After 24 h of reaction, the dry thickness of PMOXA homobrushes and P4VP homobrushes determined by VASE was 3.2 nm and 3.4 nm, respectively. The results indicated that both polymers were successfully grafted on the PDA surface. Besides, the mixed brushes also have similar thickness and are all in the range of 3.0−3.4 nm.

To further characterize the modified silicon wafers, the elemental compositions of the surfaces were determined by XPS (FIG. S4 in SM and Table I). Compared to the PDA coated surface, the modification of PMOXA led to a significant decrement in silicon signal and an increment in carbon and nitrogen signals. Moreover, the ratio of nitrogen to carbon (N/C) increased from 11.9% to 14.8%, which was attributed to a higher N/C value of MOXA than dopamine. A clear sulfur signal appeared after grafting P4VP on the PDA modified wafer. The molar proportions of sulfur element in $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, $PMOXA_{20}/P4VP_{80}$, and P4VP modified surfaces were 0.09, 0.24, 0.37, and 0.55, respectively, which were almost proportional to the molar feed ratio of P4VP. Furthermore, the N/C value of the surfaces containing

DOI:10.1063/1674-0068/cjcp2106103

TABLE I The atomic percentages of elements on the bare and modified silicon surfaces based on XPS and the corresponding thickness on the PDA-coated silicon surfaces.

	Element mole percent/%					Molar ratio/%	G_{PMOXA}/G_{P4VP}[a]	Thickness/nm
	Si	C	O	N	S	N/C		
PDA	11.10	55.04	27.32	6.54	0	11.9	/	16.1±0.4
PMOXA	6.13	65.02	19.19	9.66	0	14.8	/	3.2±0.2[b]
$PMOXA_{80}/P4VP_{20}$	1.76	71.66	16.32	10.17	0.09	14.1	78/22	3.0±0.3[b]
$PMOXA_{50}/P4VP_{50}$	1.67	73.51	14.81	9.77	0.24	13.3	53/47	3.3±0.2[b]
$PMOXA_{20}/P4VP_{80}$	7.38	68.53	14.87	8.85	0.37	12.9	16/84	3.2±0.1[b]
P4VP	5.09	72.08	13.51	8.77	0.55	12.2	/	3.4±0.3[b]

[a] G_{PMOXA}/G_{P4VP} is the mass ratio of PMOXA to P4VP. Complete calculation process of PMOXA/P4VP composition is shown in Supplementary materials.
[b] Thickness of polymer brushes on the base of PDA-coated surface.

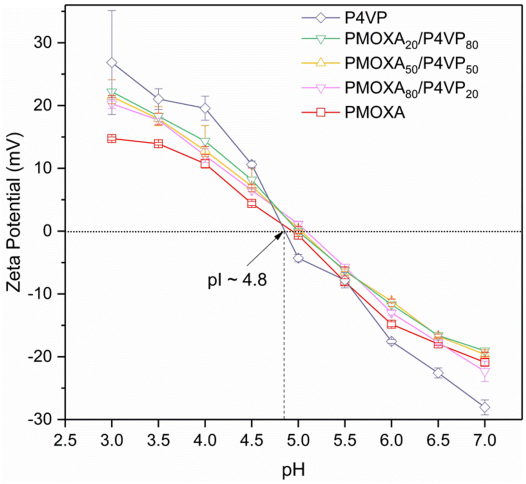


FIG. 1 Variation of surface zeta potential on polymer modified silicon wafers with different pH values.

P4VP was higher than PDA but less than PMOXA. This was caused by the fact that the N/C value of the 4VP monomer was between that of MOXA and dopamine. These results suggested the presence of two polymers in mixed brushes.

The mass ratios of PMOXA to P4VP (G_{PMOXA}/G_{P4VP}) for $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}$/ $P4VP_{50}$, $PMOXA_{20}/P4VP_{80}$ obtained by the high-resolution data of N 1s (FIG. S5 in SM) were 78/22, 53/47, and 16/84, respectively (Table S2 in SM), suggesting that the composition of the binary mixed brushes can be tailored by adjusting their percentage in the mixture of PMOXA and P4VP polymer solutions used for the preparation of the coating.

Considering the fact that the surface charge on the coating plays a significant role in the interaction of interface and protein, the zeta potential of the polymer modified surfaces at different pH values was investigated. As shown in FIG. 1 and FIG. S6 (in SM), the change of zeta potential of PDA, PMOXA, P4VP, and PMOXA/P4VP mixed brushes modified silicon wafers with pH value (from positive to negative) show similar trend. Although the mechanism of PDA formation has not yet been clearly demonstrated [38], it is clear that PDA is rich in phenolic and amino groups [39]. When pH is lower than 4.5, positive charge on PDA modified silicon wafers might result from protonated amino groups; while negative zeta potential value at pH higher than 4.5 is mainly due to the dissociation of phenolic groups [40, 41]. These results are in agreement with previous studies on pH-dependent zeta potential of PDA microspheres which showed a trend of zeta potential from positive to negative with increasing pH value [40]. For PMOXA homobrushes, lower positive charge at lower pH (pH<5) was mainly due to the enhanced interfacial hydronium ion concentration; and the negative zeta potential obtained at higher pH (pH>5) was probably due to the specific affinity of anions to PMOXA chains [22, 42, 43]. The zeta potential of P4VP reached 0 mV around pH 4.8, consistent with the isoelectric point of the P4VP mentioned in Ref.[31]. When pH is below 4.8, P4VP is protonated, and the positive zeta potential increased with increasing P4VP content in the binary mixed brushes at the same pH value, indicating that the more protonated P4VP there was, the more positive charges on the surface. Meanwhile, negative potential observed at pH above 4.8 for P4VP or mixed brushes coating might be due to the affinity of environment OH^- and Cl^- [43]. Similar results were also observed by Houbenov

DOI:10.1063/1674-0068/cjcp2106103

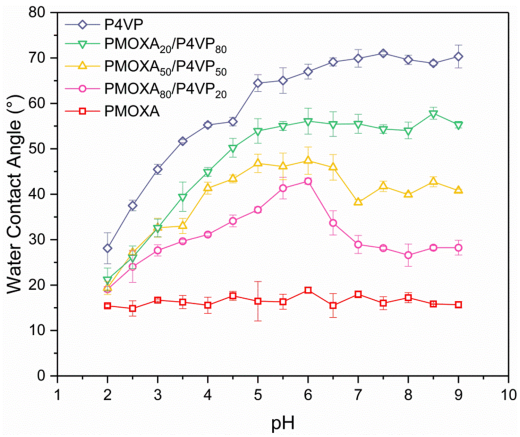


FIG. 2 Water contact angle plotted as a function of pH for PMOXA, $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, $PMOXA_{20}/P4VP_{80}$, and P4VP brushes modified surfaces.

et al. [44] in their research about the zeta potential of poly(2-vinylpyridine) (P2VP, a kind of polymer similar to P4VP) coated silicon wafer.

B. Switchable ability of modified substrate

WCA is used to estimate the wettability of material surfaces. FIG. 2 shows the change of WCA on PMOXA, $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, $PMOXA_{20}/P4VP_{80}$, and P4VP brushes modified surfaces along with the variation of pH. The WCA value of PMOXA-modified surfaces was around 16° from pH=3 to pH=9, displaying the excellent hydrophilicity and negligible pH sensitivity of PMOXA. For P4VP-modified surfaces, the WCA value increased dramatically from 28° to 67° with increasing pH value from 2 to 6, and then rose slightly until pH=9. For mixed brushes modified surfaces, the WCA was enhanced when the content of P4VP in the mixed brushes at same pH value increased. Compared with P4VP homobrushes, WCA of PMOXA/P4VP mixed brushes modified surfaces showed a similar trend in the range of pH=2 to pH=6. Interestingly, when pH changed from 6 to 7, the WCA of mixed brushes modified surfaces decreased and the degree of decrease (ΔWCA) was different for mixed brushes modified surfaces (0.6° for $PMOXA_{20}/P4VP_{80}$, 9.2° for $PMOXA_{50}/P4VP_{50}$, and 14.0° for $PMOXA_{80}/P4VP_{20}$, Table S3 in SM). The degree of decrease increased with increasing composition of PMOXA in the mixed brushes. This may be attributed to the mechanism of conformational changes of P4VP in response to pH. At lower pH value (pH<4.8) P4VP chains were highly protonated and stretched away from the surface [45, 46], which led to longer P4VP chains occupying the outermost surface in the mixed brushes and played a dominant role (the theoretical contour chain lengths of P4VP and PMOXA were 23.90 nm and 10.62 nm, respectively) (Table S1 in SM). However, when pH increased to 7, PMOXA chains were on the top and played dominant role due to the collapse of P4VP chain, which made the surface hydrophilic. Besides, more PMOXA content in mixed brushes caused more hydrophilic surface. FIG. S7 (in SM) shows the repeated tests in WCA caused by repeatedly switching conditions (from pH=3 to pH=7) for the silicon surfaces modified by $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, and $PMOXA_{20}/P4VP_{80}$. The SD lower than 5° in five cycles indicated the stably reversible transformation of the hydrophilicity/hydrophobicity for the silicon surfaces modified by the $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, and $PMOXA_{20}/P4VP_{80}$. The stable and controlled wettability of the PMOXA/P4VP mixed brushes will have a great potential in controlling adsorption and desorption of proteins.

C. Switchable behaviour of the brushes toward pepsin adsorption

Pepsin, produced in the gastric mucosa of vertebrates, is a reliable indicator for the diagnosis of laryngopharyngeal reflux disease [34], therefore in this work pepsin was chosen as a model protein to study the interaction between surfaces and proteins. In order to find the optimum conditions for efficient adsorption and desorption of pepsin on the polymer modified surface, the adsorption of pepsin on the P4VP homobrushes modified gold sensor was studied by SPR under different pH (FIG. S8 in SM). According to the SPR results, pH=3 and pH=7 were chosen as the protein adsorption and desorption conditions, respectively in the work followed (the reasons are stated in Supplementary materials).

The switching performance of brushes to pepsin adsorption was evaluated by fluorescence microscope using FITC-pepsin. The adsorption of FITC-pepsin at pH=3 and pH=7 was investigated on bare and polymer brushes modified glass wafers, the results are shown in FIG. 3. Strong fluorescence was observed on both bare glass substrate and PDA coating surface at pH=3, and the intensity of fluorescence slightly decreased after both of them were treated by the deionized water (pH=7). After being modified by PMOXA, little fluorescence was detected on the surface at both pH val-

DOI:10.1063/1674-0068/cjcp2106103

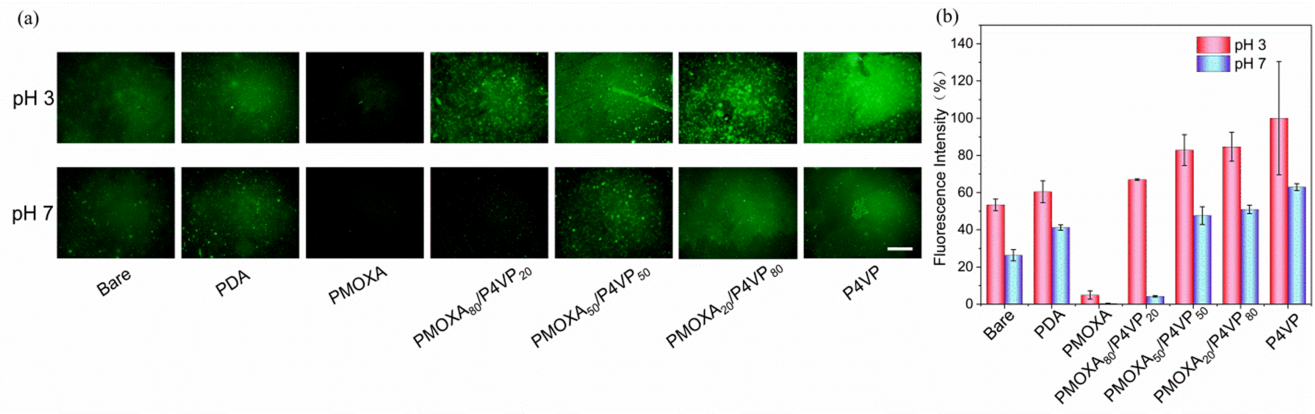


FIG. 3 (a) Fluorescence images of bare and modified glass wafers immersed in the FITC-pepsin solution of pH=3 for 3 h, and the images of the corresponding samples immersed in the deionized water (pH=7) for another 2 h (bars are 50 μm). (b) The corresponding relative fluorescence intensity of the fluorescence images when the P4VP modified glass wafer control was normalized to 100% at pH=3.

ues showing pH independent protein resistant property of PMOXA. For P4VP coated substrate, obvious fluorescence was observed at pH=3, whereas, there was a 37.0% decline in fluorescence intensity at pH=7. These results suggested that the desorption percentage for P4VP coated substrates is limited since only 37.0% of adsorbed pepsin departs from the P4VP brushes upon desorption. For PMOXA/P4VP mixed brushes modified surfaces, the introduction of PMOXA caused a significant reduction in fluorescence intensity when the condition changes from pH=3 to pH=7. Meanwhile, the desorption percentage increased with increasing PMOXA ratio in mixed brushes modified surfaces from $PMOXA_{20}/P4VP_{80}$ to $PMOXA_{80}/P4VP_{20}$ modified surfaces. Among them, 93.6% of the adsorbed pepsin could be desorbed from $PMOXA_{80}/P4VP_{20}$ modified surfaces when pH changed from 3 to 7, displaying an excellent switchable-property toward protein adsorption.

Mixed polymer brushes in our work included two components with different performances, and they performed their functions in different manners. As shown in FIG. 4, at pH=3, P4VP chains are highly protonated and stretched away from the surface (the positive charge on the surface was enhanced by increasing P4VP percentage in the binary mixed brushes at pH=3). They would occupy the outmost layer of the film owing to their longer contour chain length than that of PMOXA (the theoretical contour chain lengths of P4VP and PMOXA were 23.90 nm and 10.62 nm, respectively). Therefore, the protein adsorption happened because of the electrostatic interaction between positively charged

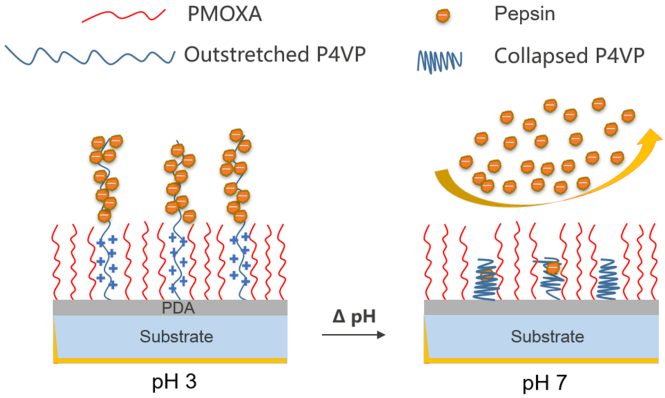


FIG. 4 Schematic representation of pepsin adsorption/desorption on the PDA anchored PMOXA/P4VP brushes modified surface.

P4VP and negatively charged pepsin; at pH=7 (higher than the isoelectric point of P4VP), the deprotonation of ionizable groups caused the collapse of P4VP chains, resulting in partial desorption of pepsin; meanwhile, PMOXA chains are on the top of the coating (the WCA values at pH=7 decreased with increasing the PMOXA fractions in PMOXA/P4VP mixed brushes), which could further repel the adsorption of pepsin. Therefore, the amount of pepsin adsorbed on PMOXA/P4VP mixed brushes could be tuned through adjusting pH value of the solution in contact with the surface as well as the fraction of PMOXA and P4VP in the mixed brushes.

In order to further study the pepsin adsorption/desorption process quantitatively, *in situ* SPR experiments were carried out. As shown in FIG. 5, the pH=3 solution first passed through the sensor for the

DOI:10.1063/1674-0068/cjcp2106103

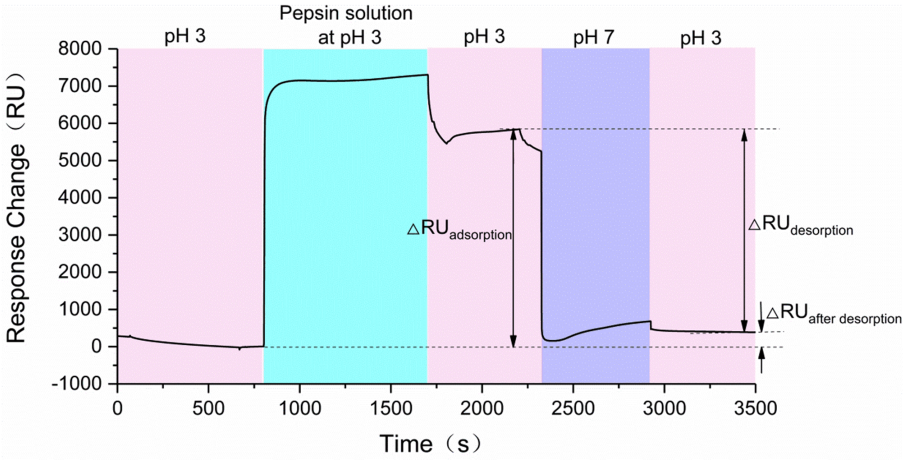


FIG. 5 SPR sensorgram of pepsin adsorption at pH=3 and release at pH=7 on the PDA anchored $PMOXA_{80}/P4VP_{20}$ modified surface.

TABLE II Hydrated mass per unit area calculated from SPR data after the adsorption of pepsin at pH=3 and after its possible desorption at pH=7, as well as the resultant desorption percentage.

Sample	$\Delta m_{\text{adsorption}}/(\text{ng/cm}^2)$	$\Delta m_{\text{after desorption}}/(\text{ng/cm}^2)$	Desorption percentage/%
Bare	252.8	246.8	2.4
PDA	256.1	164.2	35.9
PMOXA	113.6	0.5	99.6
$PMOXA_{80}/P4VP_{20}$	582.6	44.2	92.4
$PMOXA_{50}/P4VP_{50}$	827.7	320.2	61.3
$PMOXA_{20}/P4VP_{80}$	851.8	342.0	59.8
P4VP	971.8	471.4	51.5

baseline signal, and then the pepsin solution (pH=3, 0.1 mg/mL) was injected for the protein adsorption procedure. Finaly, the deionized water (pH=7) was flowed through the sensor surface to enable the desorption of pepsin. The results determined by SPR measurement are presented in Table II (the hydrated mass per unit area existed on the gold sensor after adsorption ($\Delta m_{\text{adsorption}}$), then after desorption ($\Delta m_{\text{after desorption}}$) from a series of bare or modified sensors). The desorption percentage was also presented:

$$\text{Desorption percentage} = \frac{\Delta m_{\text{adsorption}} - \Delta m_{\text{after desorption}}}{\Delta m_{\text{adsorption}}} \times 100\% \quad (1)$$

SPR data show that pepsin adsorbed on bare and PDA coating at pH=3 were 252.8 ng/cm^2 and 256.1 ng/cm^2, respectively. Insignificant amount of pepsin was desorbed from bare SPR sensor and 35.9% of the pepsin desorption occurred on the PDA coating. Once PMOXA homobrushes were grafted to PDA coating, the amount of pepsin adsorption at pH=3 dropped dramatically and almost all pepsin were desorbed after the deionized water (pH=7) passing through. The results showed that PMOXA had excellent anti-pepsin property, which was ascribed to its outstanding hydrophilicity. Whereas, P4VP modified surface displayed entirely different performance, which adsorbed 971.8 ng/cm^2 of pepsin at pH=3, while only had a desorption percentage of 51.5% at pH=7.

For PMOXA/P4VP mixed brushes modified gold sensors, the hydrated mass of pepsin adsorption on $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, and $PMOXA_{20}/P4VP_{80}$ brushes modified surfaces were 582.6, 827.7, and 851.8 ng/cm^2, respectively. The desorption percentage of $PMOXA_{80}/P4VP_{20}$, $PMOXA_{50}/P4VP_{50}$, and $PMOXA_{20}/P4VP_{80}$ brushes modified surfaces were 92.4%, 61.3%, and 59.8%, respectively. It is observed that PMOXA/P4VP mixed brushes could achieve a larger amount of pepsin adsorption than PMOXA homobrushes at pH=3 and a higher desorption capacity at pH=7 than P4VP homobrushes. Among these three mixed brushes,

DOI:10.1063/1674-0068/cjcp2106103

$PMOXA_{80}/P4VP_{20}$ coated sensor exhibited the highest desorption efficiency which was fairly consistent with fluorescence microscopy results.

IV. CONCLUSION

Binary mixed brushes composed of PMOXA and P4VP with a variety of compositions were fabricated through PDA as anchor in this work. The composition of the binary mixed brushes can be tailored by simply adjusting mass proportion of both polymers in grafting solution. The charge density on the coating surface was influenced by the fraction of P4VP in mixed brushes and the pH of the solution. While at pH=3, the zeta potential could increase with the increment of P4VP fraction in the mixed brushes, and positively charged P4VP chains would occupy the outermost surface of the mixed brushes; while at pH=7, PMOXA chains would occupy the top of the surface, and the hydrophilicity of the surface increased with increasing the PMOXA fraction in the mixed brushes. The amounts of pepsin adsorbed on the mixed brushes could be controlled through regulating pH value of the surroundings and composition of the mixed brushes. Increasing P4VP content led to more pepsin adsorption, while more PMOXA content resulted in higher desorption efficiency. When the feed ratio of PMOXA and P4VP was 80/20, the obtained PMOXA/P4VP mixed brushes ($PMOXA_{80}/P4VP_{20}$) coated surface not only absorbed large amounts of pepsin at pH=3, but also exhibited the highest desorption efficiency (>92%) at pH=7. Therefore, this switchable coating could pre-concentrate the proteins and then release it rapidly, which could improve the sensitivity of protein detections, and has a great potential for diagnosis of protein-marked diseases.

V. ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No.21674102).

[1] M. Hedayati, M. M. Reynolds, D. Krapf, and M. J. Kipper, ACS Appl. Mater. Interfaces **10**, 31892 (2018).
[2] H. Kono, F. Otaka, and M. Ozaki, Carbohydr. Polym. **111**, 830 (2014).
[3] J. Moeller-Siegert, J. Parmentier, K. Anselme, and C. Vix-Guterl, J. Mater. Sci. **48**, 3722 (2013).
[4] G. Bharath, D. Prabhu, D. Mangalaraj, C. Viswanathan, and N. Ponpandian, RSC Adv. **4**, 50510 (2014).
[5] X. R. Liu, C. Pan, and Y. M. Wang, Chin. J. Chem. Phys. **32**, 497 (2019).
[6] Y. Xu, Y. Engel, Y. Yan, K. Chen, D. F. Moyano, P. L. Dubin, and V. M. Rotello, J Mater. Chem. B **1**, 5230 (2013).
[7] P. Roach, D. Farrar, and C. C. Perry, J. Am. Chem. Soc. **128**, 3939 (2006).
[8] A. Bratek-Skicki, V. Cristaudo, J. Savocco, S. Nootens, P. Morsomme, A. Delcorte, and C. Dupont-Gillain, Biomacromolecules **20**, 778 (2019).
[9] A. Levin, S. Cinar, M. Paulus, J. Nase, R. Winter, and C. Czeslik, Biophys. Chem. **252**, 106194 (2019).
[10] M. Kumakura and I. Kaetsu, J. Mater. Sci. **21**, 3179 (1986).
[11] B. Wu, Y. Q. Chu, Z. Y. Dai, and C. F. Ding, Chin. J. Chem. Phys. **21**, 217 (2008).
[12] L. J. Liang, Q. Wang, T. Wu, J. W. Shen, and Y. Kang, Chin. J. Chem. Phys. **22**, 627 (2009).
[13] F. Mumtaz, C. S. Chen, H. K. Zhu, M. Atif, and Y. M. Wang, Chin. J. Polym. Sci. **36**, 1328 (2018).
[14] D. Zhao, R. Rajan, and K. Matsumura, ACS Appl. Mater. Interfaces **11**, 39459 (2019).
[15] E. Psarra, U. König, Y. Ueda, C. Bellmann, A. Janke, E. Bittrich, K. J. Eichhorn, and P. Uhlmann, ACS Appl. Mater. Interfaces **7**, 12516 (2015).
[16] A. Kroning, A. Furchner, D. Aulich, E. Bittrich, S. Rauch, P. Uhlmann, K. J. Eichhorn, M. Seeber, I. Luzinov, S. M. Kilbey, B. S. Lokitz, S. Minko, and K. Hinrichs, ACS Appl. Mater. Interfaces **7**, 12430 (2015).
[17] A. Bratek-Skicki, Polymers (Basel) **12**, 660 (2020).
[18] M. F. Delcroix, G. L. Huet, T. Conard, S. Demoustier-Champagne, F. E. Du Prez, J. Landoulsi, and C. C. Dupont-Gillain, Biomacromolecules **14**, 215 (2013).
[19] N. Adams and U. S. Schubert, Adv. Drug Deliv. Rev. **59**, 1504 (2007).
[20] W. B. Tsai, Y. H. Chen, and H. W. Chien, J. Biomater. Sci. Polym. Ed. **20**, 1611 (2009).
[21] J. Svoboda, O. Sedlacek, T. Riedel, M. Hruby, and O. Pop-Georgievski, Biomacromolecules **20**, 3453 (2019).
[22] T. He, D. Jańczewski, S. Jana, A. Parthiban, S. Guo, X. Zhu, S. S. Lee, F. J. Parra-Velandia, S. L. Teo, and G. J. Vancso, J. Polym. Sci., Part A: Polym. Chem. **54**, 275 (2016).
[23] L. N. Xiang, L. J. Chen, L. Tan, C. Zhang, F. H. Cao, S. T. Liu, and Y. M. Wang, Chin. Chem. Lett. **24**, 597 (2013).
[24] L. Tauhardt, M. Frant, D. Pretzel, M. Hartlieb, C. Bücher, G. Hildebrand, B. Schröter, C. Weber, K. Kempe, M. Gottschaldt, K. Liefeith, and U. S. Schubert, J. Mater. Chem. B **2**, 4883 (2014).

DOI:10.1063/1674-0068/cjcp2106103

[25] L. Tauhardt, K. Kempe, M. Gottschaldt, and U. S. Schubert, Chem. Soc. Rev. **42**, 7998 (2013).
[26] C. Pan, X. R. Liu, K. Gong, F. Mumtaz, and Y. M. Wang, J. Mater. Chem. B **6**, 556 (2018).
[27] K. Gong, C. Pan, K. He, H. K. Zhu, L. J. Chen, M. X. Hou, and Y. M. Wang, J. Appl. Polym. Sci. **136**, (2019).
[28] N. Sahiner and O. Ozay, React. Funct. Polym. **71**, 607 (2011).
[29] M. X. Wang, F. Yan, L. Z. Zhao, Y. H. Zhang, and M. Sorci, RSC Adv. **7**, 1687 (2017).
[30] L. Wu, U. Glebe, and A. Boker, Macromolecules **49**, 9586 (2016).
[31] J. Clara-Rahola, A. Moscoso, A. Belen Ruiz-Muelle, M. Laurenti, P. Formanek, J. M. Lopez-Romero, I. Fernandez, J. F. Diaz, J. Rubio-Retama, A. Fery, and R. Contreras-Caceres, J. Colloid Interface Sci. **514**, 704 (2018).
[32] Y. Li and Y. Sun, J. Chromatogr. A **1373**, 97 (2014).
[33] J. Yang, X. J. Loh, B. H. Tan, and Z. Li, Macromol. Rapid Commun. **40**, e1800013 (2019).
[34] E. Ocak, G. Kubat, and I. Yorulmaz, Balk. Med. J. **32**, 46 (2015).
[35] J. T. Lai, D. Filla, and R. Shea, Macromolecules **35**, 6754 (2002).
[36] G. Somaratne, F. Nau, M. J. Ferrua, J. Singh, A. Ye, Dupont, R. P. Singh, and J. Floury, Food Hydrocolloids **98**, 105258 (2020).
[37] Y. Hou, J. Jiang, K. Li, Y. Zhang, and J. Liu, J. Phys. Chem. B **118**, 1962 (2014).
[38] J. Svoboda, M. Král, M. Dendisová, P. Matějka, and O. Pop-Georgievski, Colloids Surf. B: Biointerfaces **205**, 111897 (2021).
[39] S. Hong, Y. S. Na, S. Choi, I. T. Song, W. Y. Kim, and H. Lee, Adv. Funct. Mater. **22**, 4711 (2012).
[40] Q. Liu, B. Yu, W. Ye, and F. Zhou, Macromol. Biosci. **11**, 1227 (2011).
[41] M. X. Hou, M. Zhang, L. J. Chen, K. Gong, C. Pan, and Y. M. Wang, Talanta **202**, 426 (2019).
[42] H. K. Zhu, F. Mumtaz, C. Zhang, L. Tan, S. T. Liu, Y. Zhang, C. Pan, and Y. M. Wang, Appl. Surf. Sci. **426**, 817 (2017).
[43] R. Zimmermann, U. Freudenberg, R. Schweiss, D. Kuttner, and C. Werner, Curr. Opin. Colloid Interface Sci. **15**, 196 (2010).
[44] N. Houbenov, S. Minko, and M. Stamm, Macromolecules **36**, 5897 (2003).
[45] P. Marcasuzaa, M. Save, P. Gerard, and L. Billon, J. Colloid Interface Sci. **581**, 96 (2021).
[46] Y. W. Harn, Y. J. He, Z. W. Wang, Y. H. Chen, S. Liang, Z. L. Li, Q. Li, L. Zhu, and Z. Q. Lin, Macromolecules **53**, 8286 (2020).

DOI:10.1063/1674-0068/cjcp2106103